REPORT ON RUTIN IN TABLETS

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A method for the determination of rutin in pharmaceutical tablets was included in the 9th Edition of the National Formulary (1). This method was derived from the tentative spectrophotometric procedure of Porter, *et al.* (2).* A revision of the method, made to detect and allow for non-rutin absorption, has now been given a collaborative test in 19 laboratories and is the subject of this report. Two progress reports have been made previously to the A.O.A.C. (3).

The method tested collaboratively depends on the quantitative extractability of rutin from tablets by acidified ethanol, and on the fact that the maximum at 352.5 mμ in the characteristic absorption spectrum can provide a precise measure of the rutin concentration. The solvent chosen for the extraction was 50 per cent ethanol containing 5 per cent acetic acid. The inclusion of acid in the extraction reagent is important because rutin decomposes in alkaline media and solutions of many of the tablet preparations are alkaline. Figure 1 shows the absorption spectrum of rutin in acidified water. The curve, with two absorption maxima, is characteristic of the flavonols. In the case of rutin these maxima are at 255 and 352.5 mμ. The location and intensity of the maxima, especially the long wavelength maximum, depend on both solvent and *p*H. The long wavelength maximum moves toward longer wavelengths and increases in intensity when the *p*H exceeds 6. Repeated analyses of rutin† at this laboratory have established its absorptivity at 352.5 mμ as 26.3 at low *p*H in water solution. Under the conditions of the procedure to be outlined, the absorption by rutin follows the Lambert-Beer Law.

* A modification of the method of Porter appeared in *J. Pharm. Pharmacol.*, 1, 323 (1949) by R. V Swann.

† The rutin used in this study was specially prepared by Dr. J. Naghski. The purity of the sample was established by repeated crystallizations and subsequent analysis by ultraviolet absorption (2).

To detect absorption by contaminants, the solution absorbance, defined by $A = \log (1/T)$, where T is the transmittance relative to solvent, is measured at the wavelength of maximum absorption by rutin, 352.5 mμ, and at two wavelengths, 338.5 mμ and 366.5 mμ, equidistant from the maximum. Absorbance ratios $R_1 = A_{338.5}/A_{352.5}$, and $R_2 = A_{366.5}/A_{352.5}$ are then determined. A consideration of Figure 2 will explain this approach. When observations are made on pure rutin the ratios are $R_1 = 0.914$ and $R_2 = 0.842$. If R_1 is increased and R_2 is decreased an interfering absorption of negative slope is indicated. If the change in the ratios is reversed it indicates that the interfering absorption has a positive slope. If both ratios are increased it indicates a non-selective or nearly uniform interference. Quercetin is a natural contaminant in rutin preparations and is

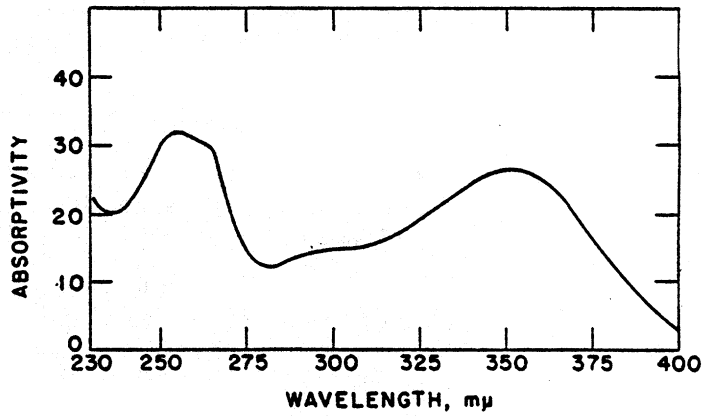


FIG. 1.—Absorption spectrum of pure rutin in acidified water.

permissible up to a level of 5 per cent (1). Structurally, quercetin differs from rutin only in that it lacks the glucose-rhamnose residue attached to the rutin molecule. Its absorption spectrum differs from that of rutin in that the long wavelength maximum is at 366.5 instead of 352.5 mμ (which impelled the choice of wavelengths used in R_2) and its absorptivity is approximately twice as great. With quercetin as the interfering constituent, R_1 is decreased and R_2 is increased. The quantities of rutin and quercetin present may be calculated by solving simultaneous equations based on known constants of rutin and quercetin and absorptivities observed at two wavelengths. Limits were calculated for R_1 and R_2, assuming wavelength errors of ± 0.2 mμ and an observational error of ± 0.002 absorbance units. The calculated range was ± 0.009. However, the collaborative study indicates that the values should be: for R_1, ± 0.009 and for R_2, ± 0.013.

The method was applied to the analysis of 42 preparations supplied by

20 tablet manufacturers.* The results may be summarized as follows: 23 preparations (55 per cent) had ratios indicative of essentially pure rutin; 12 preparations (29 per cent) had ratios indicating the presence of quercetin; in 6 preparations (14 per cent) R_1 exceeded its limits, whereas R_2 remained within its limits. The last condition was interpreted as indicating an interfering absorption with a negative slope, which could be ignored. Figure 2 illustrates this type of interference and the magnitude of the error created by neglecting it. Of the 42 preparations analyzed, only one had ratios outside both allowable limits. Thirty-four preparations (81 per cent) had a rutin content within the ± 7.5 per cent of the labeled amount of rutin permitted by the National Formulary (1). Four preparations gave values below 92.5 per cent of the labeled amount of rutin. Recovery experiments on synthetic samples, which included the usual tablet excipients, gave values within ± 2 per cent of those anticipated. Believing that no simple method could be expected to cover completely all the possibilities of tablet formulation, but that the procedure proposed would indicate the majority of significant interferences, the Associate Referee offered the method for criticism at the 1951 Association meeting. Since there were no major criticisms, the method was subjected to collaborative study.

THE COLLABORATIVE STUDY

The main points to be determined by collaborative study were the validity of the value, 26.3, as the absorptivity of rutin at 352.5 mμ, the values of the ratios, R_1 and R_2, and their limits, and the precision and accuracy to be expected from the procedure.

A single weighed sample of pure rutin was supplied to each collaborator, with instructions for its transfer and dilution. No extraction manipulations were required. This sample was included to provide a check on the wavelength and photometric scales. Should any data submitted be definitely irregular, these two important factors could be evaluated and the suspected data interpreted in the light of these facts.

The second section of the study required the recovery of known amounts of pure rutin. This step called for the extraction of rutin from a powdered preparation which included some of the usual tablet excipients (lactose, starch, calcium phosphate). Efforts to prepare a synthetic tablet or powder of known rutin content were abandoned after repeated attempts produced preparations which gave low recoveries. This difficulty was

* The following manufacturers supplied tablet samples: Abbott Laboratories, North Chicago, Ill.; Boyle and Co., Los Angeles; S. F. Durst & Co., Inc., Philadelphia; Eli Lilly & Co., Indianapolis; Keith-Victor Pharmacal Co., St. Louis; The S. E. Massengill Co., Bristol., Tenn.; Pitman-Moore Co., Indianapolis; Premo Pharmaceutical Laboratories, Inc., South Hackensack, N. J.; Schenley Laboratories, Inc., New York; Sharp & Dohme, Inc., Philadelphia; E. R. Squibb & Sons, Brooklyn; R. J. Strasenburgh Co., Rochester, N. Y.; U. S. Vitamin Corp., New York; Buffington's, Worcester, Mass.; Paul B. Elder Co., Bryan, Ohio; Empire Chemical Co., New Brunswick, N. J.; National Drug Laboratories, Inc., Chicago; Richlyn Laboratories, Inc., Philadelphia; Raymer Pharmacal Co., Philadelphia; and Standard Chemical Co., Des Moines, Iowa.

traced to the preferential adsorption of rutin on the walls of the containers used. The added rutin could easily be recovered if the entire sample (including container washings) was analyzed, but if portions of the whole were used, the results were low. To overcome this difficulty, samples of pure (unknown to the collaborator) rutin were supplied, with instructions for weighing and admixture of excipients. Enough rutin and excipients was supplied for quadruplicate analyses. This step was to check the absorptivity value, the ratios and their limits, and to indicate the precision and accuracy to be expected.

The third section of the study called for the analysis of a commercial tablet. The tablet chosen contained ascorbic acid as an additional active ingredient. The rutin used in the preparation of the tablet showed evidence of quercetin. The source of the tablet was unknown to the collaborators but the labeled value of 20 mg per tablet was given. The analysis of this tablet provided a test of the method under more extreme conditions than in the preceding section.

Instructions, data sheets, and a questionnaire were supplied to collaborators in addition to samples for analysis. The instruction sheet described the procedure and calculations and also requested the analysis of other commercial samples. The data sheets had designated blanks for the recording of original data and the intermediate and final calculations. This form of reporting allowed recomputation of the results submitted. The questionnaire included questions on wavelength calibration, absorption cells, grade of glassware used, and questions on the utility and acceptability of the method. Twenty-three sets of samples were distributed to the 14 manufacturers and 9 government laboratories that cooperated.* Four collaborators had to withdraw from the study due to personnel changes or instrumental difficulties.

METHOD

EQUIPMENT AND REAGENTS

(a) *Centrifuge tubes.*—Conical, 50 ml.

(b) *Centrifuge.*—With head accommodating 50-ml tubes.

(c) *Glass stirring rods.*—Of small enough diam. to dislodge material from the tips of 50 ml conical centrifuge tubes.

(d) *Glass funnels.*—Approx. 45 mm diam., short stem.

(e) *Water bath.*—70–80°C.

(f) *Flasks.*—Volumetric; 100 ±0.4 ml or better; 250 ±1.0 ml or better; 500 ±2.0 ml or better.

(g) *Transfer pipets.*—10 ±0.04 ml or better.

(h) *Spectrophotometer.*—Capable of isolating the following wavelengths: 338.5 mμ, 352.5 mμ, and 366.5 mμ.

* The following laboratories participated in the collaborative study: Abbott Laboratories, North Chicago, Ill.; Boyle and Co., Los Angeles; Eli Lilly and Co., Indianapolis; S. E. Massengill Co., Bristol, Tenn.; Premo Pharmaceutical Laboratories, Inc., South Hackensack, N. J.; Schenley Laboratories, Inc., New York; Sharp and Dohme, Inc., Philadelphia; R. J. Strasenburgh Co., Rochester, N. Y.; U. S. Vitamin Corp., New York; The Upjohn Co., Kalamazoo, Mich.; Food & Drug Administration Laboratories in Philadelphia, San Francisco, Cincinnati, Denver, Chicago and New York; Department of National Health and Welfare, Food and Drug Divisions, Ottawa, Canada; and Eastern Regional Research Laboratory, U. S. Dept. of Agriculture, Philadelphia.

(i) *Absorption cells.*—Matched, 1 cm.

(j) *Analytical balance.*—Accurate to ±0.5 mg.

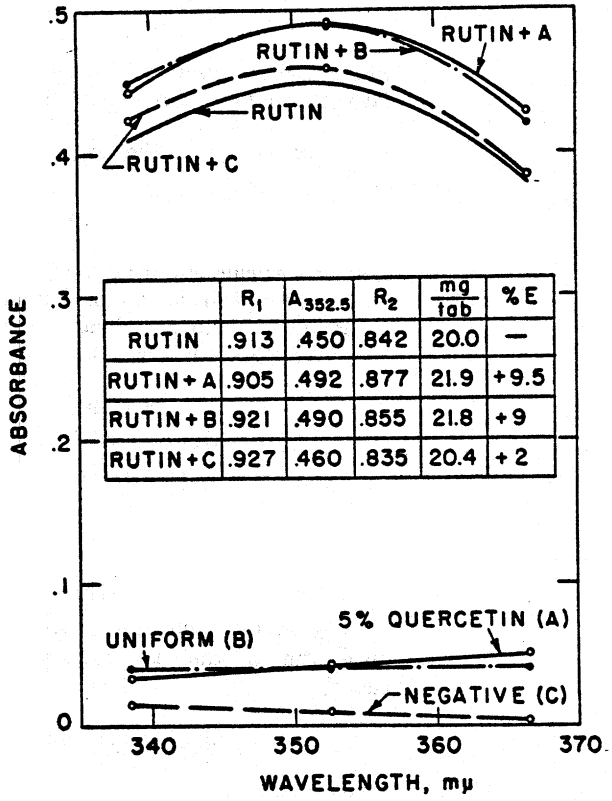
(k) *Acetic acid.*—Glacial, A.C.S.

(l) *Ethanol.*—U.S.P., 95%.

(m) *Acid-alcohol reagent.*—Prepared with above reagents; 550 ml 95% ethanol plus 50 ml glacial acetic acid dild to one l with distd H_2O.

DETERMINATION

Extraction.—Weigh directly into a 50 ml centrifuge tube the number of tablets required to give 0.05 to 0.50 g of rutin (not less than 5 tablets). Record the number and wt. (If tablets are coated, after weighing dissolve coating with distd H_2O, discard the aq. washings, and transfer the rutin-contg core to centrifuge tubes.) Add 20 ml acid-alcohol reagent and, by means of the stirring rod, break up tablets. After tablets are thoroly disintegrated, heat mixt. in H_2O bath maintained at



	R_1	$A_{352.5}$	R_2	mg/tab	%E
RUTIN	.913	.450	.842	20.0	—
RUTIN + A	.905	.492	.877	21.9	+9.5
RUTIN + B	.921	.490	.855	21.8	+9
RUTIN + C	.927	.460	.835	20.4	+2

FIG. 2.—Effect of absorbing impurities on the absorbance curve of rutin near the maximum at 352.5 mμ. The lower group of curves illustrate background absorbance where: (A) has a positive slope, as exampled by quercetin; (B) is uniform with wave length; and (C) has a negative slope. The upper group of curves show how these impurity absorptions modify the absorbance curve of rutin. The table gives numerical data on the absorbance by pure rutin and the three impure rutin preparations; the ratios R_1 and R_2; the computed weight of rutin in mg per tablet; and, in the last column, the per cent error brought about by ignoring the effect of the absorbing impurities.

70–80°C. for 10 min. During this period resuspend the material occasionally by stirring. At the end of this period remove stirring rod, rinse with acid-alcohol reagent, and centrifuge mixt. at ca 2000 r.p.m. for 15 min. After centrifugation, decant supernatant into 250 ml volumetric flask. Use funnel, decant with one smooth motion, and allow tube to drain for ca 10 sec. While still inverted, rinse mouth of tube with acid-alcohol reagent. Ext. twice more, starting with "Add 20 ml acid-alcohol reagent. . . . " After the third extn, dil. combined supernatants to 250 ml with acid-alcohol reagent. Any insol. material may be removed by filtration after diln if the first portions of filtrate are discarded. Depending on the original wt of rutin taken, make a diln with distd H_2O to give a final concn of 0.01–0.03 g/l of rutin. Precipitates forming during this aq. diln may be removed by filtration if the first portions of filtrate are discarded. Discarding the first 15–20 ml of filtrate guards against concn changes due to adsorption.

ABSORPTIMETRY

Det. the absorbance of this aq. diln at 338.5, 362.5, and 366.5 mμ against a distd H_2O blank. Calc. the following:

$$a_{362.5} = \frac{A_{362.5}}{bc}$$

where a = absorptivity; A = absorbance; b = cell length, cm; and c = concn in g/l (at the final diln, assuming the tablet to be completely soluble).

$R_1 = \frac{A_{338.5}}{A_{362.5}}$, the ratio of the absorbancies at 338.5 and 352.5 mμ.

$R_2 = \frac{A_{366.5}}{A_{362.5}}$, the ratio of the absorbancies at 366.5 and 352.5 mμ.

A sample calculation, using typical data, is as follows:

No. of tablets, 5. Wt. of 5 tablets, 812 mg. Av. wt. of tablet, 162.4 mg. Sample wt., 0.812 g. Final diln, 10–200 ml. Cell length $b = 1.004$ cm. Concn $c = 0.812 \times 4 \times 10/200 = 0.1624$ g/l.

$A_{338.5} = 0.490$ $\qquad R_1 = \frac{0.490}{0.537} = 0.913$

$A_{362.5} = 0.537$

$A_{366.5} = 0.453$ $\qquad R_2 = \frac{0.453}{0.537} = 0.844$

$$a_{362.5} = \frac{0.537}{1.004 \times 0.1624} = 3.293$$

CALCULATION OF RUTIN CONTENT

If $R_1 = 0.914 \pm .009$ and $R_2 = 0.842 \pm 0.013$, the extd material can be considered pure rutin and the wt of rutin per tablet can be calcd by means of the following equation:

$$\text{mg rutin per tablet} = \frac{a_{362.5}}{26.3} \times \text{av. wt. of tablet (mg)}$$

Using the data given in the above sample calculation,

$$\text{mg rutin per tablet} = \frac{3.293}{26.3} \times 162.4 = 20.3$$

A value of R_1 beyond its upper limit while R_2 remains within its range indicates an interfering absorption which diminishes rapidly enough to be ineffective at 352.5

mμ. Under this condition the absorbance observed at 352.5 mμ is accepted as correct and the rutin content calcd as for pure rutin. An increase in R_2 while R_1 remains within or below its limits usually indicates the presence of quercetin. The amount of rutin and quercetin may be calcd as follows:

$$\text{mg rutin per tablet} = [0.1452a_{352.5} - 0.1273a_{366.5}] \times \text{av. wt. of tablet (mg)}$$

$$\text{mg quercetin per tablet} = [0.05099a_{352.5} + 0.06057a_{366.5}] \times \text{av. wt. of tablet(mg)}$$

The value of $a_{366.5}$ is calcd in the same manner as $a_{352.5}$ except that $A_{366.5}$ is used. The above equations are based on $a_{352.5}$ and $a_{366.5}$ for rutin as 26.31 and 22.15, respectively, and for quercetin, 55.29 and 63.06, respectively.

A simultaneous increase or decrease of both ratios beyond their respective limits indicates an invalidating condition. This condition could be due to an interfering absorption or it may indicate destruction of the rutin in the tablet formulation. Interpretation of results requires that the analyst use reasonable judgment based on all the facts; the above limiting conditions are intended only as guides.

RESULTS OF THE COLLABORATIVE STUDY

The results of the study are collected in Table 1. All the results reported were recomputed by the Associate Referee and then computed independently by a colleague. Where errors in calculations were discovered, the specific point was investigated and the result reported in the table was verified.

The Standard Sample.—This was the sample of pure rutin used to test the instrument. Since only single determinations were made on weighed samples, only the average values for the 19 analysts are reported: $R_1 = 0.907$, $R_2 = 0.845$, and $a_{352.5} = 26.47$. No significant errors were demonstrated by the data submitted on this sample.

The Recovery Sample.—This was the sample which required weighing and extraction. The data indicate that the mean of quadruplicate determinations for $a_{352.5}$ will fall within 26.31, ± 0.52, or that 95 times in 100 the error should not exceed 2 per cent. The most probable values for R_1 and R_2 are 0.914 ± 0.009, and 0.842 ± 0.013, respectively, where the limits represent two times the standard deviation of the means for R_1 and R_2. Some indication of the accuracy of the method can be obtained by comparison of the two values for the absorptivity at 352.5 mμ. The value 26.47 was obtained by simple dilution and 26.31 by extraction.

The Tablet Sample.—The factors evaluated by this sample are the constancy of the tablet weight, the limits for R_1 and R_2, and the combined precision of the tablet composition and the method. The tablet weights were constant to ± 0.5 per cent. The limits for R_1 and R_2 are, as they should be, essentially the same as determined from the Recovery Sample. The precision is not as high as in the Recovery Sample, the standard deviation being 2.7 per cent as compared to 1 per cent. Inhomogeneity of sample rules out commercially prepared tablets as standards for evaluating precision. This variation from tablet to tablet is reflected in the generally higher standard deviations of the individual analysts. The precision of the method is better evaluated by the Recovery Sample because, under

TABLE 1.—*Compilation of data submitted by collaborators*[a]

COLLABORATOR NO.	RECOVERY SAMPLE						TABLET SAMPLE							
	R_1		R_2		$s_{R_1-R_2}$		AV. WT TABLETS		R_1		R_2		MG/TAB	
	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s
15	9172	19	8432	28	2610	24	1639	7	9075	13	8635	34	1942	16
17	9134	26	8432	41	2661	13	1641	11	8969	20	8667	12	1941	25
18	9171	16	8358	7	2595	6	1644	4	9117	20	8610	40	2064	7
34	9157	23	8293	34	2650	51	1632	5	9092	16	8459	33	2080	24
35	9121	16	8399	10	2666	8	1639	10	9055	34	8627	18	1961	28
36	9081	32	8451	3	2584	7	1644	11	9000	24	8659	30	1924	21
37	9146	18	8428	41	2638	7	1641	4	9067	15	8574	10	2023	10
55	9194	42	8441	38	2633	16	1639	9	9015	9	8616	20	1994	25
56	9121	13	8476	16	2645	27	1636	11	9054	8	8683	7	1941	17
57	9063	16	8457	12	2605	14	1643	1	9045	4	8675	4	1918	4
73	9176	18	8399	31	2628	10	1637	3	9037	2	8636	23	1946	13
74	9217	31	8435	42	2665	8	1635	6	9144	9	8640	8	1970	6
75	9108	16	8513	36	2645	16	1640	4	9040	7	8751	0	1915	2
76	9108	14	8297	24	2580	8	1644	7	9052	20	8516	29	2031	36
77	9229	59	8441	53	2633	25	1636	0	9096	29	8617	8	1970	9
93	9057	13	8370	9	2618	6	1630	7	9163	25	8564	95	1975	51
95	9119	33	8541	17	2632	20	1648	6	9010	35	8718	30	1897	26
96	9127	51	8440	42	2655	17	1644	4	9072	44	8712	36	1884	36
97	9148	34	8365	24	2640	6	1640	8	9095	10	8583	17	2025	12
$\bar{\bar{x}}$	9139		8419		2631		1640		9063		8634		1968	
$s_{\bar{x}}$	47		63		26		5		49		71		55	

[a] Values given to the nearest integer. Standard deviation, s; $s = \sqrt{\frac{\sum (x - \bar{x})^2}{n - 1}}$; x = individual values; $\bar{x}$ = mean of the individual values; n = number of analyses (in this case, $n = 4$); $\bar{\bar{x}}$ = average of the means; $s_{\bar{x}}$ = standard deviation of the means ($n = 19$). For ease in calculations, decimal points have been shifted. See sample calculation for magnitude.

the conditions established, the problem of homogeneity of sample was avoided.

The Questionnaire.—The replies to questions concerning the instrument used may be summarized as follows: all the collaborators used the Beckman DU spectrophotometer; 10 used the tungsten lamp plus filter; 9 used the hydrogen lamp; nearly all checked the wavelength scale by means of a mercury lamp; the majority used high sensitivity settings and slit widths in the neighborhood of 0.3 mm.

None of the collaborators felt the method required equipment that was not already part of their general laboratory equipment. No operation was considered particularly troublesome. A few collaborators made suggestions for minor changes, e.g. "warming tablets hastens disintegration," "use sintered glass funnels instead of centrifugation," "use glass stoppered centrifuge tubes," "use sand as a diluent to avoid gumming." A few were confused by the calculations and asked for elucidation. All felt that the qualitative indications of interference made evident by the values of R_1 and R_2 were worth while. The method was considered acceptable by all the collaborators and nearly all who tried the method on their own preparations or other commercial preparations reported success. The exception was a complex tablet preparation containing four active ingredients plus dyes.

RECOMMENDATION

It is recommended* that the proposed method be adopted, First Action.

ACKNOWLEDGMENT

The Associate Referee would like to thank the manufacturers and collaborators for their fine cooperation and to acknowledge the technical assistance of Miss Mary Ann Morris and Mr. Charles S. Fenske. Thanks are due Dr. William L. Porter, previous Associate Referee and author of the National Formulary procedure, for advice during the early phases of the work, and Dr. Joseph Naghski for supplying the pure rutin sample.

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* For report of Subcommittee B and action of the Association, see *This Journal*, **36**, 52 (1953)